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Seasonal Variations of Indoor Microbial Exposures and Their Relation to Temperature, Relative Humidity, and Air Exchange Rate

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Indoor microbial exposure has been related to adverse pulmonary health effects. Exposure assessment is not standardized, and various factors may affect the measured exposure. The aim of this study was to investigate the seasonal variation of selected microbial exposures and their associations with temperature, relative humidity, and air exchange rates in Danish homes. Airborne inhalable dust was sampled in five Danish homes throughout the four seasons of 1 year (indoors, $n = 127$; outdoors, $n = 37$). Measurements included culturable fungi and bacteria, endotoxin, *N*-acetyl-beta-D-glucosaminidase, total inflammatory potential, particles (0.75 to 15 μm), temperature, relative humidity, and air exchange rates. Significant seasonal variation was found for all indoor microbial exposures, excluding endotoxin. Indoor fungi peaked in summer (median, 235 CFU/ m^3) and were lowest in winter (median, 26 CFU/ m^3). Indoor bacteria peaked in spring (median, 2,165 CFU/ m^3) and were lowest in summer (median, 240 CFU/ m^3). Concentrations of fungi were predominately higher outdoors than indoors, whereas bacteria, endotoxin, and inhalable dust concentrations were highest indoors. Bacteria and endotoxin correlated with the mass of inhalable dust and number of particles. Temperature and air exchange rates were positively associated with fungi and *N*-acetyl-beta-D-glucosaminidase and negatively with bacteria and the total inflammatory potential. Although temperature, relative humidity, and air exchange rates were significantly associated with several indoor microbial exposures, they could not fully explain the observed seasonal variations when tested in a mixed statistical model. In conclusion, the season significantly affects indoor microbial exposures, which are influenced by temperature, relative humidity, and air exchange rates.

Indoor microbial exposure has been related to adverse pulmonary health effects, headache, and allergy (6, 40, 45, 57). However, lack of a standardized sampling methodology has made it difficult to compare data between studies and to ultimately relate exposure levels to health effects when examining the data across different studies. Potential seasonal variation of microbial exposures also adds to the difficulty in comparing data across studies, as different studies may choose to perform their sampling during different seasons of the year.

Seasonal variation has been shown for fungal genera, including *Cladosporium*, *Penicillium*, and *Aspergillus*, and for bacteria, particularly the Gram-positive species of the phylum *Firmicutes*, in indoor air (9, 15, 43, 46). It is therefore also reasonable to consider the influence of parameters that are dependent on the season on indoor microbial concentrations. Such parameters include the temperature and relative humidity (RH) of the air, both indoors and outdoors. In fact, the relative humidity has been shown to have profound effects on spore and particle release from fungal structures with which surfaces are infested (26, 39). Denmark is located in northern Europe and experiences a temperate climate. The use of ventilation and air-conditioning systems is not common, and thus, people normally ventilate their homes by opening windows. The air exchange rate (AER) of Danish homes may therefore also be dependent on the season, as one can expect more open windows during the summer. Since microbial exposures may have different sources, both indoors and outdoors, the AER may also be influential on indoor microbial levels.

Exposure to fungi; bacteria, including the actinomycetes; and endotoxin has been linked to pulmonary health effects. Therefore, these microbial agents were quantified in this study. Fungal genera typically found within the indoor environment are sources of po-

tent allergens and inflammogens (22), and several epidemiological studies have coupled fungal exposure with asthma, allergy, and sick building syndrome (5, 8, 21, 45, 56). Exposure to bacteria has been associated with blocked nose and eye symptoms (14). In particular the spore-forming Gram-positive actinomycetes have been shown to be involved in the development of lung diseases, and *Streptomyces albus* was shown to be a direct cause of hypersensitivity pneumonitis (20). Endotoxin is a proinflammatory lipopolysaccharide originating from the cell wall of Gram-negative bacteria and has been shown to induce airway inflammation and respiratory disorders (16, 30, 37, 47, 50). *N*-Acetyl- β -D-glucosaminidase (NAGase) is a chitinase thought to be produced by all chitin-containing fungi (10), including many fungal genera, such as *Penicillium*, *Aspergillus*, *Alternaria*, and *Trichoderma*, commonly found in indoor dust (1). Higher activity of NAGase has been found in homes of patients with sarcoidosis (53), and NAGase can stimulate cells to interleukin-8 secretion (1).

We have developed a cell-based assay that can measure inflammatory effects of various microbial factors simultaneously. The assay has previously been used on bioaerosol samples from biofuel plants (55) and on dust samples from homes (7). It has been shown that cell activation could be related to the multifactorial

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TABLE 1 Characteristics of the five homes

Home ^a	Type	Construction yr	No. of occupants ^b	No. of pets	Floor area (m ²)	No. of rooms	Ventilation type ^c	Known moisture/fungal problems
A	Detached house	1964	2/1	1 dog	130	5	Natural	No
B	Detached house	1921	2/2	2 cats	143	5	Natural	No
C	Town house	2007	2/0	None	104	4	Natural	No
D	Detached house	1947	2/0	None	190	4	Natural	Yes
E	Apartment, 2nd floor	2004	1/0	None	90	3	Mechanical	No

^a All homes are situated in urban areas within a 40-km radius of Copenhagen.

^b Number of adults/children.

^c Natural ventilation is provided by thermal, wind, or diffusion effects through doors, windows, or other intentional openings in the building. Mechanical ventilation is provided by mechanically powered ventilation systems, such as fans and blowers.

composition of the bioaerosol and could thus be used as a measurement of the total inflammatory potential (TIP) of a given sample (55). The TIP was therefore measured in this study to obtain a combined inflammatory response of the total microbial load from airborne dust samples collected from the homes. Particle (0.75 to 15 μm) concentrations and the mass of inhalable dust were also measured to study possible associations between microbial exposures and particles of different origins and because particle mass has been linked with symptoms of the airways (14).

The aim of this study was to investigate the seasonal variation of the above-mentioned airborne microbial exposures, TIP, inhalable dust, and particle (0.75 to 15 μm) concentrations in five Danish homes. Associations between the measured indoor exposures were examined, and the relationships between the levels of outdoor and indoor exposures were analyzed for fungi, bacteria, endotoxin, and inhalable dust. Lastly, the influences of air temperature, relative humidity (indoors and outdoors), and air exchange rates on concentrations of indoor microbial exposures were investigated.

MATERIALS AND METHODS

Selection of homes. As part of a study conducted by the Center for Indoor Air and Health in Dwellings (CISBO) (48), five homes were selected for intensive investigation of their indoor environments throughout four seasons of 1 year (April 2010 to March 2011). Airborne dust measurements from these homes have been reported in another study that compared various dust-sampling methods (7). Therefore, details regarding the choice of homes can be found elsewhere (7). Home characteristics are displayed in Table 1.

Sampling of airborne dust. Active air sampling on filters was used for sampling of airborne dust. Gesamtstaubprobenahme (GSP) inhalable-dust samplers were mounted with polycarbonate filters (37 mm; pore size, 1.0 μm ; GE Water and Process Technologies) or Teflon filters (37 mm; pore size, 1.0 μm ; Millipore). Polycarbonate filters (indoors, $n = 127$, and outdoors, $n = 37$) were used for quantification of bacteria and fungi. Teflon filters (indoors, $n = 127$, and outdoors, $n = 37$) were used for

weighing of inhalable dust, quantification of endotoxin and NAGase activity, and measurement of TIP in the granulocyte assay. The samplers were hung 1.5 m above floor level in the kitchens, bedrooms, living rooms, and bathrooms of the homes, in addition to outdoors. Aerosols were aspirated at an airflow of 3.5 liters per minute, which was verified or adjusted every 1 to 2 h during the average 6-h sampling period. Sampling was performed according to the scheme in Fig. 1. The sampling was conducted during the daytime, and for the most part, the occupants of the homes were not home; thus, activity during the sampling time was mainly generated by members of the CISBO project team. Therefore, the activity levels during each sampling day were presumed to be more or less equal. Furthermore, all windows of the homes were kept closed during sampling. Both external and internal doors of the homes were also kept closed during sampling and were only opened for entrance into or exit from the homes or rooms.

Extraction of dust. Polycarbonate filters were extracted in 5.0 ml pyrogen-free solution (0.05% Tween 80 and 0.85% NaCl) by orbital shaking (500 rpm for 15 min at room temperature). Teflon filters were extracted in 5.0 ml pyrogen-free 0.05% Tween 20 by orbital shaking (300 rpm for 60 min at room temperature). Extraction of dust took place no later than 24 h after sample retrieval.

Quantification of microorganisms. Microorganisms were quantified using a modified CAMNEA (collection of airborne microorganisms on Nuclepore filters, estimation and analysis) method (36). The fungi culturable on dichloran glycerol agar (DG 18 agar; Merck, Germany) at 25°C were counted after 3 and 7 days of incubation. Mesophilic bacteria and actinomycetes were quantified after 3 and 7 days of incubation on 100% and 10% nutrient agar (Oxoid, Basingstoke, United Kingdom) with actidione (cycloheximide; 50 mg/liter), respectively. Culture analyses were performed directly after extraction of the dust from the filters. The limit of detection (LOD) based on the mean sampled air volume was 4 CFU/m³.

Quantification of endotoxin. Samples used for endotoxin quantification were centrifuged (1,000 $\times g$) for 15 min. The supernatant was analyzed in duplicate for endotoxin, using a chromogenic kinetic *Limulus* amoebocyte lysate test (Kinetic-QCL endotoxin kit; Lonza Walkersville Inc.). A standard curve obtained from an *Escherichia coli* O55:B5 standard endotoxin solution was used to determine the concentration of endo-

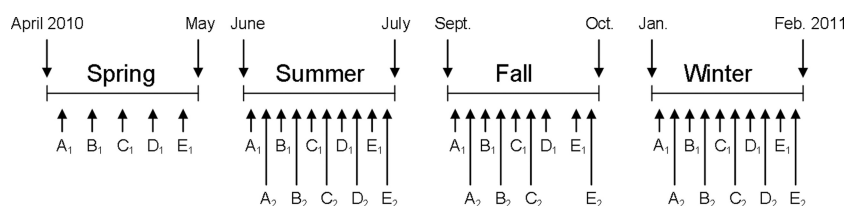


FIG 1 Sampling scheme. Each season represents 5 weeks. The letters (A to E) represent the five homes, and the subscript numbers represent day 1 or day 2 sampling. There are 7 days between days 1 for different homes and 4 days between day 1 and day 2 for the same home. Due to logistical reasons, no day 2 sampling took place during spring or fall for home D.

toxin. The standard endotoxin solution has been assayed against the International Standard for Endotoxin, and its potency is expressed in endotoxin units (EU) ($12.0 \text{ EU} \approx 1.0 \text{ ng}$). The LOD based on the mean sampled air volume was 0.021 EU/m^3 .

Quantification of NAGase activity. NAGase activity was quantified by an assay described previously (32) with minor modifications. In brief, 100 μl of 200 μM 4-methylumbelliferyl (MUF) *N*-acetyl- β -D-glucosaminide (the MUF substrate) (Sigma) was added to 1 ml 50 mM Tris-maleate buffer (pH 5). Addition of 50 μl dust sample suspension was followed by vortexing and incubation at 25°C for 30 min. The enzymatic reaction was stopped by adding 1.9 ml ice-cold 96% ethanol. The tubes were then centrifuged for 5 min ($2,600 \times g$; 2°C), and 900 μl of the supernatant was added to 100 μl 2.5 M Tris buffer to reach pH 10. After brief vortexing, 200 μl of this solution was added to a black microtiter plate in replicates of 3. Fluorescence derived by the release of 4-methylumbelliferone (4-MU) was detected at an emission wavelength of 446 nm and an excitation wavelength of 377 nm by a fluorescence spectrometer. NAGase activity was calculated by comparing the sample fluorescence with that of a standard curve containing 4-MU (0 to 7,095 pmol/ml). The LOD was difficult to establish, as the standard curve varied from day to day. Thus, we considered the LOD to be the lowest measured concentration (2.27 pmol 4-MU/ml or 9.35 pmol 4-MU/ m^3).

Measurement of the TIP. Measurement of the TIP was conducted using the granulocyte assay, an assay that was developed mainly for the purpose of assessing microbial contamination of medicines (54). The assay is based on the differentiated HL-60 cell line, which, upon exposure to microbial compounds, reacts by producing reactive oxygen species (ROS) quantifiable by a luminal-dependent chemiluminometric assay. In this study, measurement of TIP was conducted as described previously (55). Prior to analysis, all samples were subjected to ultrafiltration for removal of Tween, as Tween inhibits the cellular ROS response in the granulocyte assay. Ultrafiltration was also conducted as described previously (55). The chemiluminescence reaction caused by sample activity was measured with a thermostated (37°C) Orion II Microplate luminometer (Berthold Detection Systems, Germany), which measured relative light units (RLU) per second for 1 s every 120 s for 180 min. For every sample, the accumulated RLU/s was calculated by summing the RLU/s measurements throughout the 180-min period. To account for day-to-day variation in the reactivity level of the cells, the accumulated RLU/s value was normalized to that of endotoxin, 0.5 EU/ml, from the endotoxin standard curve (0 to 20 EU/ml), which was included in each run.

Measurement of inhalable dust. The concentration of airborne inhalable dust was measured gravimetrically; thus, when inhalable dust is mentioned throughout this paper, we are referring to the mass of the inhalable dust. Teflon filters were kept at constant temperature (22°C) and relative humidity (50%) for at least 16 h before being weighed, both pre- and postsampling. Three extra filters (blanks) were always transported to and from the homes, together with the filters used for sampling. The average mass of the three blanks was then subtracted from the mass of each of the filters used for sampling. To establish LODs, we used three times the standard deviation of 10 blanks divided by the mean sampled volume. The LOD for inhalable dust was 0.007 mg/m^3 .

Particle measurement. The concentration of airborne particles was measured numerically; thus, when particles are mentioned throughout this paper, we are referring to the number of particles. The Grimm Portable Dust Monitor (model 1.109; Grimm Technologies, Inc. Douglasville, GA) was used to measure airborne particles (0.75 to 15 μm ; one measurement per minute) in the living rooms of the homes during the sampling time. The particles quantified could be subfractionated into different size ranges of aerodynamic diameter (d_a). We chose to focus on two fractions of particles able to enter the thoracic region (particulate matter below 10 μm [PM_{10}]): a lower size range (0.75 to 2 μm), which is part of the respirable fraction ($\text{PM}_{2.5}$), and a larger size range (2 to 10 μm).

Measurement of temperature and relative humidity. Air temperature and relative humidity were measured indoors using Tinytag Plus

Data Loggers (Gemini Data Loggers, United Kingdom). The loggers were placed in close proximity to GSP samplers and set to measure once every 5 min for 15 min between 10:00 and 11:00 am on each sampling day. The mean value from the 15-min measurement period was used. Outdoor temperature and relative humidity measurements were obtained from the Danish Meteorological Institute. The meteorological stations conducting the measurements were situated within 15 km of each home. Median values from measurements conducted every 10 min from 9 am to 4 pm during the day of sampling were used. The four 5-week periods in which the sampling was conducted represented the typical four seasons for the Danish climate, and the outdoor temperatures for each period differed significantly (see Table 5).

Measurement of AER. AERs were continuously measured in each home over a 2- to 4-day period following "day 1" of the airborne dust sampling during the four seasons (Fig. 1). The measurements were performed using constant concentration methods with a target level of 4 ppm of Freon. The concentration of tracer gas was monitored using an Innova Multi-Gas Monitor Type 1302 and an Innova Multipoint Sampler and Doser 1303 (Lumasense Technologies, Santa Clara, CA). The concentration of tracer gas was separately controlled in different rooms of each home. Whenever possible, the instruments were located behind closed doors in a room that was not directly investigated in the experiment. This was done in order to minimize potential leakage of tracer gas from the measurement setup. However, prior to the experiments, the instruments were tested for leakage, which appeared to be negligible. The average overall AER for an entire home was calculated as the total airflow entering the home, as measured by the instrument (the sum of airflows into all measured rooms), divided by the total volume of the home.

Statistical analyses. The MIXED procedure COVTEST (which provides statistical inferences for each covariance parameter) of the statistical computer program SAS (version 9.2) was used to determine the seasonal variation of all measured exposures, in addition to temperature, RH, and AERs. Season was included as a fixed effect, and day, home, and room were included as random effects. Between-home variation was determined by the *P* value corresponding to the random effect: home. Likewise the same mixed model was used to determine the effects of temperature, RH, and AER on indoor microbial concentrations, where temperature, RH, and AER were included separately as fixed effects, either alone or in combination with the season. Similarly, the effects of outdoor microbial exposures on indoor microbial exposures were determined by the same model, using concentrations of outdoor exposures as fixed effects. Paired *t* tests and Pearson correlations were also conducted using SAS (version 9.2). For exposure measurements below the LOD, the LOD divided by the square root of 2 was used. This was done in accordance with the study by Hornun and Reed (17), as our data were not highly skewed (the geometric standard deviation was below 3 for all measured exposures). The concentrations of all airborne exposures were always log transformed to achieve normalization. *P* values below 0.05 were considered statistically significant.

RESULTS

Seasonal variation of airborne exposures. The concentrations of indoor and outdoor airborne exposures, including microbial exposures, TIP, inhalable dust, and particles (0.75 to 15 μm , 0.75 to 2 μm , and 2 to 10 μm), measured during the four seasons are presented in Table 2. Seasonal variation was significant for all measured exposures, apart from indoor endotoxin, outdoor bacteria, and outdoor inhalable dust (Table 2). As two outliers from fall contained extremely high levels of *Wallembia sebi* organisms of unknown origin, they were deleted from the data set. There was no significant difference between homes for any of the measured indoor exposures.

A matrix correlating all indoor exposures is presented in Table 3. Regarding microbial exposures, bacteria correlated positively

TABLE 2 Indoor and outdoor airborne exposures and TIP measured during four seasons

Exposure type	Unit/m ³	Spring			Summer			Fall			Winter		
		Median (n)	Range (% > LOD)	Significance	Median (n)	Range (% > LOD)	Significance	Median (n)	Range (% > LOD)	Significance	Median (n)	Range (% > LOD)	Significance
Indoor fungi	CFU	126 (19)	Bd-311 (5)	b	235 (38)	42-1,781	a	155 (32)	41-1,687	a	26 (38)	Bd-257 (3)	c
Outdoor fungi	CFU	63.6 (5)	40.8-366	b	1,001 (10)	42.0-2,934	a	197 (9)	126-2,173	ab	18 (10)	Bd-87 (20)	c
Indoor bacteria	CFU	2,165 (19)	96-21,946	a	240 (38)	6-3,601	b	1,141 (32)	41-6,467	a	465 (38)	Bd-2,017 (3)	b
Outdoor bacteria	CFU	43.8 (5)	Bd-204 (20)	a	24 (10)	10.5-286	a	25 (9)	Bd-42.0 (33)	a	7.6 (10)	Bd-181 (50)	a
Indoor actinomycetes	CFU	Bd (19)	Bd-414 (68)	c	60 (38)	Bd-630 (24)	b	204 (32)	Bd-1,671 (19)	a	42 (38)	Bd-4,141 (24)	bc
Indoor endotoxin	EU	1.24 (19)	0.078-8.32	a	1.48 (11)	0.32-3.38	a	0.88 (10)	0.59-4.59	a	0.86 (12)	0.21-2.56	a
Outdoor endotoxin	EU	1.28 (5)	0.52-4.40	a	0.32 (5)	0.11-0.39	b	0.34 (4)	0.19-0.67	b	0.15 (10)	0.044-0.68	b
Indoor NAGase ^b	pmol 4-MU	162 (8)	Bd-989 (13)	ab	671 (13)	Bd-3,379 (15)	a	22 (16)	Bd-1,291 (44)	b	Bd (19)	Bd-1,120 (58)	b
Indoor TIP ^b	RLU ^c	3.77 (10)	1.43-5.71	ab	1.77 (21)	0.74-4.61	c	2.55 (17)	2.10-3.31	a	3.23 (19)	2.62-3.87	b
Indoor inhalable dust	mg	0.048 (19)	0.0016-0.105	a	0.037 (38)	0.013-0.095	a	0.035 (32)	0.015-0.067	a	0.024 (38)	0.0022-0.086	b
Outdoor inhalable dust	mg	0.017 (5)	0.0023-0.071	a	0.020 (10)	0.011-0.029	a	0.019 (9)	0.0023-0.040	a	0.022 (10)	0.0046-0.034	a
Indoor particles ^b	no.	2.81 × 10 ⁶ (5)	0.96 × 10 ⁶ -6.39 × 10 ⁶	a	0.96 × 10 ⁶ (10)	0.42 × 10 ⁶ -1.66 × 10 ⁶	c	1.18 × 10 ⁶ (8)	0.77 × 10 ⁶ -3.42 × 10 ⁶	b	1.53 × 10 ⁶ (5)	0.96 × 10 ⁶ -3.14 × 10 ⁶	abc
0.75-2 μm	no.	2.26 × 10 ⁶ (5)	0.72 × 10 ⁶ -5.20 × 10 ⁶	a	0.72 × 10 ⁶ (10)	0.31 × 10 ⁶ -1.18 × 10 ⁶	c	0.95 × 10 ⁶ (8)	0.58 × 10 ⁶ -2.71 × 10 ⁶	b	0.12 × 10 ⁶ (5)	0.77 × 10 ⁶ -2.53 × 10 ⁶	ab
2-10 μm	no.	5.47 × 10 ⁵ (5)	2.32 × 10 ⁵ -17.2 × 10 ⁵	a	2.41 × 10 ⁵ (10)	0.89 × 10 ⁵ -4.76 × 10 ⁵	b	2.36 × 10 ⁵ (8)	1.47 × 10 ⁵ -7.10 × 10 ⁵	b	1.85 × 10 ⁵ (5)	1.13 × 10 ⁵ -5.85 × 10 ⁵	b

^a For each exposure type, values followed by the same letter are not significantly different. Bd, below the LOD. When all samples were above the LOD, % > LOD is omitted.

^b NAGase, TIP, and particles were not measured outdoors.

^c RLU represents the accumulated RLU/s over the 180-min cell response time normalized to the accumulated RLU/s resulting from endotoxin (0.5 EU/ml).

with endotoxin and actinomycetes. The TIP of the airborne dust samples correlated positively with bacteria and endotoxin and negatively with fungi and NAGase activity. Regarding associations between microbial and other exposures, positive correlations were found between inhalable dust, particles in each size range, bacteria, endotoxin, and TIP. Fungi did not correlate with either inhalable dust or particles in any of the given size ranges.

Relationships between indoor and outdoor exposures. Airborne fungi, bacteria, endotoxin, and inhalable dust were measured outdoors, in addition to indoors. For fungi, concentrations were significantly higher outdoors than indoors during summer and fall (Table 4). For bacteria, endotoxin, and inhalable dust, indoor concentrations were always higher indoors than outdoors, except during spring for endotoxin, where the difference did not reach significance (Table 4). Correlations between outdoor and indoor exposures measured during the entire year were significant for fungi and endotoxin, but not for bacteria and inhalable dust (Table 4). When further tested in a mixed statistical model, the effect of outdoor concentrations on indoor concentrations was significant for fungi ($P < 0.0001$; $n = 127$), but not for bacteria ($P = 0.30$; $n = 127$), endotoxin ($P = 0.099$; $n = 37$), or inhalable dust ($P = 0.30$; $n = 127$).

Influence of temperature, RH, and AERs on indoor microbial exposures. Significant seasonal variation was found for temperature and RH, both indoors and outdoors, as well as for AERs (Table 5). A matrix correlating each of these factors with the measured indoor microbial exposures is given in Table 6. In particular, outdoor temperature, indoor temperature, and AER correlated positively with indoor fungi and NAGase and negatively with indoor TIP. Indoor temperature and AER correlated negatively with bacteria, and indoor RH correlated positively with fungi (Table 6).

The effects of temperature, RH, and AERs on the concentrations of indoor microbial exposures were also tested in a mixed statistical model, and P values are given in Table 7. Similar to the correlations, outdoor temperature and AER had positive effects on indoor fungi and NAGase and negative effects on indoor TIP. To test whether the effect of the season on the indoor microbial exposures was mediated by temperature, RH, or AER, each of these parameters was combined with season in the same mixed statistical model. The results showed only nonsignificant effects for each parameter when combined with season, although outdoor temperature had a nearly significant effect ($P = 0.054$) on the concentrations of indoor fungi. The effect of the season also remained significant ($P = 0.025$) in combination with outdoor temperature.

DISCUSSION

Seasonal variation of airborne exposures. Significant seasonal variation was shown for all measured indoor exposures, excluding endotoxin. The clear and significant seasonal pattern for indoor bacteria, with a large decline from spring to summer, increasing again in fall, followed by a decrease toward winter, has not been shown before. In indoor air of Chicago homes, culturable bacteria were highest in summer and fall (33), whereas in Finland, only a slight yet significant difference between summer and winter bacterial levels was shown (43). Some studies have shown seasonality in bacterial and viral infections (41, 51); thus, the risk of contracting primary or secondary bacterial infections may be higher in seasons with high concentrations of indoor bacteria, which in the present study were found to be spring and fall. The seasonal pat-

TABLE 3 Correlation matrix for indoor airborne exposures and the total inflammatory potential (TIP)

Correlation ^a							
Exposure type	Bacteria	Fungi	Actinomycetes	NAGase	Endotoxin	TIP	Particles (0.75–15 μ m)
Bacteria		$r = -0.05$; $P = 0.59$; $n = 127$	$r = 0.20$; $P = 0.030$; $n = 127$	$r = -0.18$; $P = 0.19$; $n = 56$	$r = 0.30$; $P = 0.031$; $n = 52$	$r = 0.48$; $P < 0.0001$; $n = 67$	$r = 0.32$; $P = 0.0002$; $n = 127$
Fungi	$r = -0.05$; $P = 0.59$; $n = 127$		$r = 0.18$; $P = 0.050$; $n = 127$	$r = 0.19$; $P = 0.17$; $n = 56$	$r = 0.26$; $P = 0.058$; $n = 52$	$r = -0.36$; $P = 0.0031$; $n = 67$	$r = 0.15$; $P = 0.093$; $n = 127$
Actino-mycetes	$r = 0.20$; $P = 0.022$; $n = 127$	$r = 0.18$; $P = 0.050$; $n = 127$		$r = -0.22$; $P = 0.11$; $n = 56$	$r = -0.036$; $P = 0.80$; $n = 52$	$r = -0.12$; $P = 0.34$; $n = 67$	$r = -0.060$; $P = 0.506$; $n = 127$
NAGase	$r = -0.18$; $P = 0.19$; $n = 56$	$r = 0.19$; $P = 0.17$; $n = 56$	$r = -0.22$; $P = 0.11$; $n = 56$		$r = 0.12$; $P = 0.56$; $n = 27$	$r = -0.37$; $P = 0.032$; $n = 33$	$r = -0.23$; $P = 0.084$; $n = 56$
Endotoxin	$r = 0.30$; $P = 0.031$; $n = 52$	$r = 0.26$; $P = 0.058$; $n = 52$	$r = -0.036$; $P = 0.80$; $n = 52$	$r = 0.12$; $P = 0.56$; $n = 27$		$r = 0.42$; $P = 0.014$; $n = 34$	$r = 0.030$; $P = 0.81$; $n = 67$
TIP	$r = 0.48$; $P < 0.0001$; $n = 67$	$r = -0.36$; $P = 0.0031$; $n = 67$	$r = -0.12$; $P = 0.34$; $n = 67$	$r = -0.37$; $P = 0.032$; $n = 33$	$r = 0.42$; $P = 0.014$; $n = 34$		$r = 0.030$; $P = 0.81$; $n = 67$
Inhalable dust	$r = 0.32$; $P = 0.0002$; $n = 127$	$r = 0.15$; $P = 0.093$; $n = 127$	$r = -0.060$; $P = 0.50$; $n = 127$	$r = 0.23$; $P = 0.084$; $n = 56$	$r = 0.56$; $P < 0.0001$; $n = 52$	$r = 0.030$; $P = 0.81$; $n = 67$	$r = 0.51$; $P = 0.0054$; $n = 28$
Particles 0.75–15 μ m	$r = 0.57$; $P = 0.0017$; $n = 28$	$r = 0.048$; $P = 0.81$; $n = 28$	$r = -0.31$; $P = 0.11$; $n = 28$	$r = -0.18$; $P = 0.47$; $n = 18$	$r = 0.51$; $P = 0.020$; $n = 20$	$r = 0.44$; $P = 0.02$; $n = 28$	$r = 0.51$; $P = 0.0054$; $n = 28$
0.75–2 μ m	$r = 0.53$; $P = 0.0036$; $n = 28$	$r = -0.0054$; $P = 0.98$; $n = 28$	$r = -0.31$; $P = 0.11$; $n = 28$	$r = -0.20$; $P = 0.42$; $n = 18$	$r = 0.46$; $P = 0.040$; $n = 20$	$r = 0.40$; $P = 0.033$; $n = 28$	$r = 0.46$; $P = 0.013$; $n = 28$
2–10 μ m	$r = 0.61$; $P = 0.0005$; $n = 28$	$r = 0.26$; $P = 0.18$; $n = 28$	$r = -0.24$; $P = 0.22$; $n = 28$	$r = -0.12$; $P = 0.65$; $n = 18$	$r = 0.67$; $P = 0.0013$; $n = 20$	$r = 0.46$; $P = 0.014$; $n = 28$	$r = 0.60$; $P = 0.0008$; $n = 28$

^a Significant correlations are shown in boldface.

TABLE 4 Comparison of indoor and outdoor concentrations of airborne exposures, analyzed by paired *t* tests and Pearson correlations^a

Season	Fungi					Bacteria					Endotoxin					Inhalable dust				
	<i>t</i> value	<i>r</i>	<i>P</i> value ^t	<i>P</i> value ^r	<i>n</i>	<i>t</i> value	<i>r</i>	<i>P</i> value ^t	<i>P</i> value ^r	<i>n</i>	<i>t</i> value	<i>r</i>	<i>P</i> value ^t	<i>P</i> value ^r	<i>n</i>	<i>t</i> value	<i>r</i>	<i>P</i> value ^t	<i>P</i> value ^r	<i>n</i>
Spring	-1.62	0.64	0.12	0.0031	19	8.92	0.046	<0.0001	0.85	19	0.37	0.53	0.72	0.020	19	2.57	-0.075	0.02	0.76	19
Summer	-5.74	0.58	<0.0001	<0.0001	38	7.15	0.051	<0.0001	0.76	38	4.56	0.49	0.0039	0.26	7	7.30	0.23	<0.0001	0.16	38
Fall	-1.99	0.29	0.056	0.10	32	20.10	0.25	<0.0001	0.17	32	5.18	0.64	0.0066	0.25	5	5.12	0.062	<0.0001	0.74	32
Winter	2.59	0.083	0.014	0.62	38	12.59	0.23	<0.0001	0.17	38	4.23	0.17	0.0082	0.75	6	2.74	0.22	0.0093	0.18	38
Entire yr	-2.19	0.71	0.030	<0.0001	127	19.91	0.13	<0.0001	0.14	127	4.19	0.46	0.0002	0.0039	37	7.68	0.062	<0.0001	0.49	127

^a A positive *t* value, together with *P* values of <0.05, means higher levels indoors compared to outdoors. The *P* value^t and the *P* value^r are the *P* values corresponding to paired *t* tests and Pearson correlation coefficients, respectively.

tern shown for indoor fungi, peaking from spring to summer and declining throughout fall to winter, is highly comparable to the findings of other studies that also took place in urban areas, including homes in Australia (9) and Central Europe (12, 15, 43). Temporal increases in fungal spore concentrations have been associated with increases in hospitalization for asthma among children (3). Thus, in connection with asthma exacerbation, it is important to consider the seasonal variation in exposure. The concentration of indoor actinomycetes also showed seasonal dependency in our study. However, a large number of samples were below the LOD, which is a finding similar to that of Lee and colleagues, who collected bioaerosols from urban homes in Cincinnati (23). Analogous to their study, we found that the largest proportion of samples below the LOD were from springtime. Even though our study showed significant seasonal variation for outdoor endotoxin (similar to the findings of an earlier study [25]), we did not observe any significant effect of the season on indoor endotoxin levels, which is in accordance with studies conducted in Baltimore (29) and Boston (38) homes.

Despite differences in home characteristics (Table 1), no significant differences were found between homes for any of the measured microbial exposures. Supporting this result, it was found that the majority of home characteristics (including year of construction, heating system, observation of fungi, and presence of a dog) were not significantly related to concentrations of fungi in indoor air (42). Similarly, most home characteristics had no effect on endotoxin concentrations, apart from floor type and air conditioner use (29). Although home D was reported to have fungal/moisture problems, measurements of fungal exposure were not significantly higher in the home. This is in accordance with various studies showing no clear relationship between visible fungal growth and the level of airborne microbial exposure (34, 44, 49). However, other studies have in fact found higher levels of airborne fungi in buildings with moisture/fungal problems than in reference buildings (12, 18, 19). Therefore, the question of whether visible fungi on the building structure are related to mea-

sured airborne exposures is disputable and probably influenced by various factors, including the possibility of hidden fungal growth.

Associations between the different indoor exposure types. A significant correlation was found between indoor endotoxin and bacteria. However, this correlation was weak ($r = 0.3$; $P = 0.031$), indicating the presence of nonviable bacteria and/or that most bacteria measured in the homes were Gram positive. Supporting the latter possibility, it was found that the bacterial flora of indoor dust was dominated by Gram-positive species (33, 46, 52). Stronger correlations between concentrations of airborne bacteria and endotoxin have been observed in agricultural and industrial settings (24), where the proportion of Gram-negative bacteria among total bacteria may be higher.

Measurements of TIP correlated positively with indoor bacteria and endotoxin but negatively with indoor fungi and NAGase. Previously, all four exposures were shown to correlate positively with TIP in bioaerosol samples from biofuel plants (55). However, these samples contained approximately 150 times more fungi per milliliter than the indoor air samples of the present study. The level of fungi in the samples investigated here is below the detection limit of the granulocyte assay (unpublished observations). Therefore, bacteria and bacterial components were the main contributors to the TIP of the airborne dust samples.

Hargreaves and colleagues stressed the importance of investigating potential associations and interactions between nonbiological and biological aerosol particles, as such interactions could influence particle behavior in indoor air and also the effect the particles have on health (13). In our study, indoor bacteria and endotoxin both correlated significantly with indoor particles of each size range and inhalable dust indoors, whereas fungi and NAGase did not. Similarly, no significant associations between fungal spores and PM_{2.5} respirable dust were found (13). A correlation is merely an indication of a relationship between two variables and not proof of interaction or causation. However, our results may indicate that bacteria and endotoxin are more prone than fungi and NAGase to attach to other airborne particles. In

TABLE 5 Temperature, relative humidity, and air exchange rates measured during four seasons^a

Parameter	Spring			Summer			Fall			Winter		
	Median (<i>n</i>)	Range	Significance	Median (<i>n</i>)	Range	Significance	Median (<i>n</i>)	Range	Significance	Median (<i>n</i>)	Range	Significance
Outdoor temp (°C)	9.4 (5)	7.0–16.9	b	22.4 (10)	19.4–27.3	a	13.9 (9)	8.5–16.3	b	0.75 (10)	-2.8–5.5	c
Outdoor RH (%)	69.0 (5)	46.0–94.0	bc	62.0 (10)	54.0–72.0	c	79.0 (9)	65.0–95.0	b	97.0 (10)	67.0–100	a
Indoor temp (°C)	20.3 (17)	16.1–23.2	ab	24.2 (36)	19.1–28.6	a	20.1 (31)	16.1–23.1	b	19.3 (38)	13.9–23.4	b
Indoor RH (%)	51.6 (17)	40.1–81.5	b	57.3 (37)	32.1–79.8	ab	64.4 (32)	37.8–84	a	55.7 (37)	35.5–68.8	b
AER (rooms) (h ⁻¹)	0.59 (13)	0.017–2.51	b	2.03 (13)	0.058–6.06	a	0.13 (13)	0.00–0.96	bc	0.13 (13)	0.00035–1.63	c
AER (homes) (h ⁻¹)	0.69 (5)	0.11–1.95	ab	1.54 (5)	1.040–5.01	a	0.14 (5)	0.096–1.03	b	0.19 (5)	0.030–0.53	b

^a For AER (rooms), medians are calculated from individual room measurements and only from a subset of the full data set (only rooms where airborne dust was sampled were used). For AER (homes), medians are calculated from the overall AER from each entire home. Values followed by the same letter are not significantly different.

TABLE 6 Correlation matrix of temperature, relative humidity, and air exchange rates versus indoor microbial exposures and TIP

Parameter ^a	Correlation ^b			
	Bacteria	Fungi	NAGase	TIP
Outdoor temp	$r = -0.14$; $P = 0.13$; $n = 127$	$r = 0.66$; $P < 0.0001$; $n = 127$	$r = 0.50$; $P < 0.0001$; $n = 56$	$r = -0.62$; $P < 0.0001$; $n = 67$
Outdoor RH	$r = 0.030$; $P = 0.74$; $n = 127$	$r = -0.35$; $P < 0.0001$; $n = 127$	$r = -0.30$; $P = 0.026$; $n = 56$	$r = 0.35$; $P = 0.0037$; $n = 67$
Indoor temp	$r = -0.20$; $P = 0.024$; $n = 122$	$r = 0.28$; $P = 0.0020$; $n = 122$	$r = 0.36$; $P = 0.0084$; $n = 52$	$r = -0.49$; $P < 0.0001$; $n = 64$
Indoor RH	$r = 0.022$; $P = 0.81$; $n = 123$	$r = 0.32$; $P = 0.0003$; $n = 123$	$r = -0.0039$; $P = 0.98$; $n = 52$	$r = -0.097$; $P = 0.44$; $n = 65$
AER				
Rooms	$r = -0.22$; $P = 0.039$; $n = 86$	$r = 0.39$; $P = 0.0002$; $n = 86$	$r = 0.54$; $P = 0.0002$; $n = 41$	$r = -0.56$; $P < 0.0001$; $n = 66$
Homes	$r = -0.24$; $P = 0.0057$; $n = 127$	$r = 0.31$; $P = 0.0004$; $n = 127$	$r = 0.43$; $P = 0.0011$; $n = 56$	$r = -0.56$; $P < 0.0001$; $n = 66$

^a See Table 5 for an explanation of AER (rooms) and AER (homes).^b Significant correlations are shown in boldface. Actinomycetes and endotoxin did not correlate with temperature, RH, or AER and are therefore not shown.

fact, bacteria and endotoxin correlated most strongly with the larger fraction of thoracic particles (2 to 10 μm). Accordingly, it was shown that the percentage of endotoxin was highest in thoracic dust compared to respirable and PM_{10} dust in both occupational and indoor settings (27). Furthermore, bacteria have a tendency to form aggregates (28) and may therefore take on the size range of larger particles.

Relations between indoor and outdoor exposures. Concentrations of fungi were higher outdoors than indoors in spring, summer, and fall, although in spring, the difference was not significant. In winter, concentrations of fungi were higher indoors than outdoors. Moreover, correlations between indoor and outdoor fungi were highly significant during spring and summer and also when computing the data from the entire year. This indicates that the major source for indoor fungi was outdoors, except during winter, when indoor levels exceeded outdoor levels. This indication is supported by other studies, which have found similar associations between outdoor and indoor airborne fungi (9, 23, 43). In contrast, concentrations of bacteria and inhalable dust were always higher indoors than outdoors, and indoor levels did not correlate with outdoor levels for either exposure. This points to an indoor source for indoor bacteria, likely originating from the occupants of the homes. Accordingly, large amounts of human-

derived bacterial DNA sequences have been found in house dust (46, 52). Endotoxin was higher indoors than outdoors (except during spring), and there was a significant correlation between indoor and outdoor endotoxins. Interpreting whether endotoxin measured indoors has a mainly indoor or outdoor source is thus difficult. As mentioned above, the majority of bacteria measured indoors are Gram positive and human derived (33, 46, 52), which theoretically indicates a mainly outdoor endotoxin source. The higher endotoxin levels measured indoors in our study could thus be caused by an accumulation of endotoxin indoors from both outdoor and indoor sources. This may also be the reason for the lack of significant seasonal variation for endotoxin indoors as opposed to outdoors.

Effects of temperature, RH, and AERs on levels of microbial exposures and TIP. Very diverse results have been shown by a number of studies investigating associations between indoor air temperature and RH and the levels of microbial exposures indoors. In the present study, indoor temperature and RH were positively associated with airborne fungi, just as in homes in the northeast United States (42), and indoor temperature was negatively associated with bacteria, as in Cincinnati residences (11). However, contradictory results have been found by others (4, 35), which may reflect differences in geographical location. Interest-

TABLE 7 P values for the effect of season, temperature, relative humidity, and air exchange rates on levels of indoor microbial exposures and TIP

Parameter	P^a					
	Bacteria	Fungi	Actinomycetes	NAGase	Endotoxin	TIP
Season	< 0.0001	< 0.0001	0.0049	0.014	0.53	0.0001
Outdoor temp ($^{\circ}\text{C}$)	-0.34	+ < 0.0001	+0.61	+0.0027	+0.38	- < 0.0001
Outdoor RH (%)	+0.82	-0.0041	+0.73	-0.041	-0.47	+0.026
Indoor temp ($^{\circ}\text{C}$)	-0.097	+0.075	-0.34	+0.087	+0.40	-0.0015
Indoor RH (%)	+0.74	-0.80	+0.26	+0.58	+0.85	-0.19
AER (h^{-1}) ^b						
Rooms	-0.066	+ < 0.0001	-0.91	+0.0029	-0.71	- < 0.0001
Homes	-0.040	+0.0057	+0.54	+0.0095	-0.71	- < 0.0001

^a P values for the effects of season, air temperature, relative humidity, and air exchange rates on microbial and TIP levels. Significant effects are shown in boldface. -, negative estimate; +, positive estimate.^b See Table 5 for an explanation of AER (rooms) and AER (homes).

ingly, the incidence of respiratory infections has been found to be lower among people living in environments with midrange compared to low or high relative humidity (2).

Outdoor temperature and AER were positively associated with indoor fungi and NAGase but negatively associated with indoor TIP. The AER was also negatively associated with indoor bacteria. As emphasized above, our data indicate the outdoors as the major source for indoor fungi, whereas indoor bacteria mainly come from indoor sources. One can speculate that until equilibrium between indoor and outdoor air is reached, the higher the AER, the more fungi enter the home (when the outdoor temperature is favorable for the presence of fungi) and the more diluted the indoor bacterial concentrations become. To our knowledge, only very few studies have looked at associations between the AER and the levels of microbial exposures in the indoor environment. Wu and colleagues have investigated office buildings equipped with either air-handling unit (AHU) or fan coil unit (FCU) systems. In both building types, AERs were associated positively with total fungi, though strongly so in buildings with FCU systems, which contain HEPA filters, probably retaining outdoor fungi (58). In another study, no correlation was observed between fungi in settled dust and AERs in Canadian homes (31); however, settled dust may represent longer-term exposure, not necessarily associated with AERs measured on the single day of dust collection.

A noteworthy finding from the present study is that AER had a highly significant negative effect on the TIP. That is, a higher ventilation rate may lead to decreased exposure to inflammatory microbial components measured in a granulocyte assay. Apparently, the increasing fungal levels resulting from incoming outdoor air are not high enough to counterbalance the dilution of bacteria and other possible exposures affecting the TIP.

We have applied a mixed statistical model to see whether the demonstrated effects of the season on the indoor microbial exposures were mediated by air temperature, RH (indoors and outdoors), and AERs measured in this study. The only one of these parameters that was close to retaining its significant effect in combination with the season was outdoor temperature, and this applied only to indoor fungi. As the season also retained its significant effect, outdoor temperature could only partly account for the seasonal effect on indoor fungi, indicating the involvement of other unknown, potentially season-dependent factors. Such factors could be natural parameters, such as a plant life cycle or something similar.

Concluding remarks. Overall, this study has shown that the season has an influence on the concentrations of several microbial exposures, excluding endotoxin, in indoor air. Thus, the season in which sampling is conducted affects the measured exposure. Our results indicate that fungi measured indoors mainly come from outdoor sources, except during winter, whereas bacteria have indoor sources all year and correlate significantly with indoor inhalable dust and particles (0.75 to 15 μm , but strongest at 2 to 10 μm). Outdoor temperature and AER associated positively with fungi and NAGase and negatively with TIP. A negative association was also shown between AER and indoor bacteria. This suggests that an increase in AER will cause an increase of airborne fungi and a decrease of airborne bacteria, which, for the exposure levels measured in the present study, resulted in a lower inflammatory response in granulocyte cells. Whether this effect of increasing ventilation can be generalized to inflammatory responses in other cells or animal models would be relevant to study. Although tem-

perature, RH, and AER had significant effects on concentrations of indoor microbial exposures, they could not fully explain the observed seasonal variations when tested in a mixed statistical model.

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